Senescence in immunity against helminth parasites predicts adult mortality in a wild mammal

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Abstract:

Our understanding of the deterioration in immune function in old age – immunosenescence – derives principally from studies of modern human populations and laboratory animals. The generality and significance of this process for systems experiencing complex, natural infections and environmental challenges is unknown. Here, we show that late-life declines in an important immune marker of resistance to helminth parasites in wild Soay sheep predict overwinter mortality. We found senescence in circulating antibody levels against a highly prevalent nematode

worm, which was associated with reduced adult survival probability, independent of changes in body weight. These findings establish a role for immunosenescence in the ecology and evolution of natural populations.

One Sentence Summary:

Declining immune resistance to helminth parasites predicts host mortality in wild Soay sheep.

Main Text:

Demographic senescence, the decline in survival prospects and fertility with age, is well documented in wild vertebrates and is known to play an important role in the dynamics of natural populations (*1-3*). Bio-gerontologists have made great strides towards understanding the genetic and physiological processes underpinning senescence in laboratory organisms (*4-6*), but we do not yet know whether similar mechanisms drive demographic ageing under natural conditions (*1, 3*). Declining immune function in old age, or immunosenescence, is widely observed and associated with age-related increases in morbidity and mortality in laboratory rodents and humans (*7-9*). Parasites represent a major selective force in natural populations, and the ability to mount effective immune defences against them represents a critical determinant of fitness in wild animals (*10, 11*). A growing body of evidence suggests that immunosenescence also occurs in natural populations: several recent, largely cross-sectional, vertebrate field studies have documented age-related variation in immune markers across adulthood (*12*). However, the absence of large-scale longitudinal studies simultaneously measuring parasites, immunity, health and demography in the wild has limited our ability to test how declines in immune function impact ecological and

evolutionary processes (*12*). There is a similar scarcity of human studies linking within-individual changes in immune phenotype in old age with clinical outcomes (*9*).

Gastrointestinal nematode parasites are important drivers of selection in wild vertebrate systems (*13, 14*). Despite the global impact of helminth parasites (*15, 16*), the potential for senescent declines in immune-mediated resistance to helminth infection and the consequences for human, livestock and wildlife health have rarely been considered. Studies of lab mice, humans and domestic ruminants show that resistance to such worm infections is dependent on T helper type 2 (Th2) immune responses (*17*), with a key contribution from serum antibodies (*18, 19*). There is evidence from wild mammals and humans that the intensity of worm burdens may increase in later adulthood (*20, 21*), and cross-sectional studies in laboratory mice suggest Th2 function and anti-worm antibody production may be compromised in old age (*22-24*). In particular, two studies found that elderly mice had a reduced or delayed immunoglobulin-G (IgG) antibody response to worm infection (*22, 24*), suggesting this could be an important marker of immunosenescence. Deterioration in Th2-mediated immunity to worms may therefore be responsible for increasing burdens and negative health outcomes in later life, but currently longitudinal studies testing for associations among immunity, worm burden and components of fitness are completely lacking.

We used an unmanaged population of Soay sheep on the remote St Kilda archipelago in Scotland, which has been the subject of detailed study since 1985 (*25*), to test for fitness consequences of senescent declines in immunity in a wild population. First winter lamb mortality is often high in this population (*25*). Amongst individuals that survive to adulthood, mean longevity is 5.5 years for females (maximum 16 years) and 2.7 for males (maximum 10). Demographic senescence is

well-documented in this population, with female fecundity and survival of both sexes declining progressively from around 5 years (*26*). The sheep are host to a diverse community of gastrointestinal nematode parasites, including several highly prevalent strongyle species, which have been linked to gut pathology and overwinter mortality (*27*). Counts of nematode eggs from faecal samples provide an important proxy of parasite burden (*27*). Faecal egg counts (FEC) increase with advanced age in adult Soay sheep, which could reflect a loss of immune-mediated host control of parasite infection in later life (*26*).

Our previous work has established levels of plasma immunoglobulin-G binding antigen from *Teladorsagia circumcincta* (IgG-Tc), a highly prevalent worm in both wild Soay and domestic sheep, as an important marker of immunity to helminths in this system. In adult Soay sheep, plasma levels of IgG-Tc in summer are weakly correlated with other immune measures, including other antibody isotypes (IgA, IgM and IgE) binding the same Tc antigens (*28-30*). IgG-Tc levels are also negatively associated with FEC and positively associated with subsequent winter survival, independently of other humoral and cellular immune measures (*28-30*). Further analysis showed that IgG-Tc correlates strongly ($r > 0.9$) with levels of IgG binding antigen from a range of strongyle species (Methods; Fig. S1), only some of which are present on St Kilda, suggesting a high degree of cross-reactivity. We also showed that, regardless of which strongyle species is used, levels of binding by this IgG predict subsequent survival (Table S1). This implicates IgG-Tc as a potentially powerful marker of specific immunity to helminths in this study system, and motivated an in-depth study of the causes of its association with adult mortality. Using measurements of IgG-Tc by ELISA from 2215 longitudinal blood samples collected from 797 adult sheep aged ≥ 3 years over 26 years (1990 – 2015) on St Kilda (Methods; Table S2), we tested whether IgG-Tc showed

within-individual declines in later life consistent with immunosenescence, and whether such declines were associated with parasite burden, body weight and subsequent mortality.

We found senescence in our marker of resistance to nematode infection in wild Soay sheep. Levels of IgG-Tc declined with age (β = -0.006, 95%CI = -0.010–-0.002; Table S3a), but since individuals may senesce at different rates, the number of years an individual is away from death may be a better reflection of their biological ageing patterns than chronological age itself (*31*). Accordingly, we found that years before death explained more variation in IgG-Tc than chronological age (Table 1a). Levels of IgG-Tc declined as adults approached death, and the relationship was best described by a threshold function with the decline accelerating over the final year of life (Fig. 1A; Table S3b). Males had lower average levels of IgG-Tc than females (Table 1b), but there was no detectable interaction between years before death and sex, indicating that the pattern of within-individual changes in IgG-Tc was consistent between the sexes. IgG-Tc levels were highly repeatable across the adult lifetimes of sheep, with 58% of the variance in our dataset explained by individual identity (repeatability = 0.576, 95%CI = 0.542–0.609). These results show that despite consistent among-individual differences in IgG-Tc across their lifetimes, average antibody levels declined within individuals as they approached death.

Senescent declines in immunity were associated with increased subsequent mortality risk. We used a bivariate mixed-effects model to estimate the covariance between IgG-Tc measured in summer and the probability of survival over the subsequent winter at three different levels: among-individual, among-year and within-individual (see Methods; 2202 observations of 796 individuals over 26 years). Among-individual covariance captures the association between an individual's

average adult antibody level and its overall lifespan, while within-individual (or residual) covariance represents the association between the deviation in IgG-Tc from an individual's mean value and its prospects of surviving the following winter. Among-year covariance reflects associations between the population's average antibody levels and mortality rates across years. We found that covariance between IgG-Tc and survival was statistically significant only at the within-individual level, and not at either among-individual or among-year levels (Fig. 1; Table S4). The absence of any among-individual covariance reveals that consistent differences in immunity across adulthood, potentially associated with genotype or early-life environment, did not predict lifespan (Fig. 1B). However, the positive within-individual covariance indicates that individuals showing a within-individual decline in IgG-Tc had a reduced survival probability the following winter (Fig. 1C). These data show that longitudinal changes in a marker of immune resistance in later adulthood predict mortality risk in the wild, and indicate immunosenescence may play an important role in age-related declines in demographic rates in natural populations.

The within-individual association between IgG-Tc and survival remained when associations with FEC, our index of parasite burden, and body weight were accounted for (Fig. 2; Table S5). We ran a multivariate mixed model that included all four measures as response variables, and again estimated covariance among the terms at the within-individual, among-individual and among-year levels (see Methods). As expected for a marker of resistance to worm infection, FEC and IgG-Tc were negatively associated at the within-individual level (Table S5). Higher levels of IgG-Tc at both among- and within-individual levels were associated with increased body weight, and weight covaried positively with survival at all three levels (Table S5; accounting for variation in structural size (hindleg length) in models of body weight did not change our results, Table S6). We used this

multivariate approach to test for independent effects of immunity, parasite burden and weight on subsequent survival, while accounting for the inter-dependencies among these terms (analogous to a multiple regression; see Methods). We found that within-individual deviation in IgG-Tc was still a predictor of overwinter survival (Fig. 2). The independence of the immunity–survival relationship from body weight suggests that it was not mediated by variance in individual body condition or resource availability, and that late-life declines in body weight and immunity reflect separate physiological senescence pathways. This highlights the complex, multi-faceted nature of physiological senescence in wild animals, and the need for large-scale multivariate studies to understand which processes are most important for late-life fitness across taxa and ecological contexts (*32*).

Few studies to date have investigated how the immune system changes in later adulthood in response to pathogenic, chronically infective helminth parasites. Our analyses show that associations among adult infection, immunity and survival are not driven by constitutive among-individual differences, determined by genetics or early-life conditions, but rather by within-individual variation late in life linked to senescence. Studies in laboratory mice suggest the Th2 response to worm infection becomes compromised in old age, and that the host's ability to resist infection declines as a result (*22-24*). While the observed within-individual negative correlation between FEC and IgG-Tc is consistent with a resistance function for this immune marker, our multivariate models show longitudinal declines in IgG-Tc predict mortality independently of FEC, suggesting this relationship with mortality is not solely mediated by reduced worm burden. This may reflect the indirect and therefore inherently noisy relationship between FEC and actual worm burden. However, changes in host tolerance of worm infection, a process we have previously

linked to variation in host fitness in our study system (*33*) or density-independent alterations in parasite behavior in response to host physiological deterioration (e.g. helminth suppression of the immune response, (*18*)) could also explain the relationship between IgG-Tc and survival. Our results suggest that changes in the interactions between host immunity and helminth infection during adulthood could have implications for host ecological dynamics, helminth epidemiology and host-parasite co-evolution in wild vertebrates. The focus on the development of immunity to helminths in early life in humans and livestock is understandable, but our data suggest changes in host immune responses to worm infection occur in mammals during later life.

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Supplementary Materials:

Materials and Methods

Figure S1

Tables S1-S6

References (34-52)

Data supporting the results

Table 1. Models of anti-*T. circumcincta* IgG levels measured in plasma samples collected from adult Soay sheep in summer 1990–2015. a) Comparing the explanatory power of linear mixed effects models with different fixed effects structures. All models included individual, year and ELISA plate as random intercept terms. Sex was included as a two-level factor, age as a linear covariate, and years before death (YBD) as a threshold function with a break-point at 1 year (see Table S3). df indicates the number of parameters, and ΔAIC is the difference in AIC value compared to the best model (highlighted in bold). b) Fixed and random effects estimates from the best model, with 95% confidence intervals from 1000 bootstrap replicates.

a) Model selection — n = 1869 observations of 651 individuals

Fixed effects structure	**df**	**AIC**	**ΔAIC**
Null	5	-1662.46	44.63
Sex	6	-1671.53	35.56
Age	6	-1670.06	37.02
YBD threshold	7	-1703.29	3.80
Age + Sex	7	-1680.56	26.53
YBD threshold + Sex	**8**	**-1707.09**	**0.00**
YBD threshold + Age	8	-1702.79	4.30
Age * Sex	8	-1678.72	28.37
YBD threshold * Sex	10	-1703.68	3.40
YBD threshold + Age + Sex	9	-1705.38	1.71
YBD threshold + Age * Sex	10	-1704.31	2.78
YBD threshold * Sex + Age	11	-1702.01	5.08
YBD threshold * Sex + Age * Sex	12	-1700.42	6.67

b) Best model

Random effects	**Estimate**	**Lower 95% CI**	**Upper 95% CI**		
Individual	0.023	0.020	0.026		
Year	0.001	<0.001	0.003		
ELISA Plate	0.004	0.002	0.006		
Residual	0.012	0.011	0.013		
Fixed effects				**P value**	
Intercept	0.541	0.512	0.571	<0.001	***
YBD slope (final year)	0.047	0.028	0.067	<0.001	***
YBD slope (before final year)	0.006	0.002	0.010	0.004	**
Sex (males)	-0.045	-0.081	-0.008	0.016	*

Figure Legends:

Figure 1. A. Levels of circulating anti-*T. circumcincta* IgG declined as Soay sheep approached death. Points and error bars show raw data medians and standard errors (females black circles, males grey triangles), and lines show predictions from a linear mixed effects model (Table 1b) with 95% confidence intervals (grey shading). **B.** Annual overwinter survival probability was not related to an individual's mean levels of IgG-Tc measured over adulthood, as indicated by the regression slope for the among-individual effect of IgG-Tc on survival estimated from a bivariate model (Table S4). **C.** Individuals with relatively low levels of IgG-Tc compared to their average were less likely to survival the winter, as indicated by the within-individual effect of IgG-Tc on survival estimates from a bivariate model (Table S4). Points show raw data, and black lines show regression slope with 95% credible intervals shaded in grey.

Figure 2. Independent effects of anti-*T. circumcincta* IgG, body weight and faecal egg count (FEC) measured in August on overwinter survival probability in adult Soay sheep, accounting for covariance among the traits. Regression coefficients (median of the posterior distribution with 95% credible intervals) are given for the within-individual (green circles), among-individual (black triangles) and annual effects (grey squares). Within-individual deviation in IgG-Tc was predictive of survival probability, independent of the within- and among-individual variance in body weight and FEC. Effects were estimated from a multivariate model of IgG-Tc, weight (both Gaussian), FEC (Poisson) and survival probability (threshold) (see Methods; Table S5). IgG-Tc and weight were standardised prior to inclusion in the model (mean = 0, SD = 1).